

9:00 a.m.

870-3

Metalloproteinase Inhibition Prevents Diastolic Stiffening, AMP-Breakdown, and Oxypurine Accumulation in Accelerated Heart Failure

Nazareno Paolucci, Barbara Tavazzi, Roberto Biondi, Yehezkiel A. Gluzband, Mariangela Amorini, Carlo G. Tocchetti, Sonia Donzelli, Michael T. Crow, Giuseppe Lazzarino, David A. Kass, Johns Hopkins Medical Institutions, Baltimore, MD, University of Catania, Catania, Italy

Background: Recent studies indicated increased plasma oxypurine (hypoxanthine/xanthine/uric acid) levels as an independent risk factor for worsened heart failure, suggesting oxypurine potential role for diastolic dysfunction.

Methods: We used a canine model of enhanced diastolic failure (1 week AII infusion + subacute tachypacing; 250 bpm for 48 hrs; AII+P), displaying pronounced diastolic stiffening and marked metalloproteinase (MMP) activation. MMP inhibition (MMPI) prevents diastolic stiffening, without influencing collagen content/subtype or cross-linking. Here we tested whether AII+P (n=7) enhances AMP catabolism to increase cardiac nucleoside/oxypurine and diastolic stiffening, assessing the ability of MMPI to prevent these changes.

Results: AII+P raised diastolic chamber stiffness and end-diastolic pressure ~100% and markedly activated gelatinases MMP-9 and MMP-2 (abundance and *in situ* assays). With AII+P, ATP declined while AMP catabolites increased: nucleotides (inosine, adenosine) raised from 254±94 to 1700±363nmol/g (p<0.005), and oxypurines from 100±20 to 666±67 nmol/g (p<0.001). MMP inhibition (PD-166793, 5 mg/kg/day, n=9) prevented diastolic stiffening as well as MMP-9 and -2 activation, and countered the rise in both nucleotides (804±35 nmol/g, p<0.02 vs AII-P) and oxypurines (397±17, p<0.001 vs AII-P). MMPI directly inhibited *in vitro* AMP deaminase activity in a dose-dependent manner but did not affect other steps of the purine catabolism cascade. In AII+P hearts hallmarks of oxidative stress were evident: *malondialdehyde* (lipid peroxidation index) raised from 0.31±0.1 (controls) to 6.7±2.3 nmol/g (p<0.01) while antioxidant levels (ascorbate and reduced glutathione) significantly declined. However, MMPI did not mitigate AII+P-induced oxidative stress (MDA = 6.1±1.3).

Conclusions: Diastolic stiffening is associated with a rise in myocardial content of AMP-breakdown byproducts (i.e. nucleosides and oxypurines). MMP inhibition fully prevents diastolic stiffening by limiting this accumulation, directly interfering with AMP catabolic activities (i.e. AMP deaminase), independently from the extent of cardiac oxidative stress.

9:15 a.m.

870-4

Survival, Differentiation, and Contractility of Immature Cardiac Cells Implanted Into the Outer Wall of Aorta in Rats as a Step in the Development of an Auxiliary Circulatory Pump

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Purpose: We proposed to build an auxiliary circulatory pump by implanting neonatal cardiac cells into the wall of aorta in rats. As a first step, we investigated the survival, differentiation and contractility of immature cardiac cells implanted into the wall of the abdominal aorta.

Methods: Cardiomyocytes from neonatal Fischer rats (both sexes) were injected into the outer wall of the abdominal aorta at a site 3 mm above the take-off of the renal arteries in female Fischer rats. Rats were divided into 2 groups: (1) medium only (n=22); (2) neonatal cardiomyocytes (n=22, 5x10⁶ cells each). At 2 or 6 weeks, the grafted site on the aorta was exposed and fixed for histological and immunohistologic examination.

Results: At 2 weeks after transplantation, 7 out of 10 aortas in the cell group, but none of 10 in the medium group showed spontaneous rhythmic beating at the grafted site following excision of the heart. The intra-aortic pressure was comparable between the cell group and the medium group after the aorta was clamped at its origin (2.88 ± 0.45 vs 2.94 ± 0.63 mmHg, p=0.94). Pacing in a cell grafted aorta increased aortic pressure 5 fold from baseline. PCR of the SRY gene to identify male cells was positive in 3 examined aortas in the cell group confirming presence of transplanted cells and none of 3 aortas in the medium group at 6 weeks. Hematoxylin and eosin staining showed viable grafts in the outer wall of the cell-treated aortas in 9 out of 10 aortas at 2 weeks and 9 out of 9 aortas at 6 weeks; and none of the aortas receiving medium at 2 (n=10) or 6 weeks (n=9). Neonatal cardiomyocytes in the graft formed compact, longitudinally oriented cardiac muscle bundles, and were differentiated with cross striations and a high degree of vascularization. Immunohistochemical staining for sarcomeric actin was positive in 4 out of 10 aortas at 2 weeks and 9 out of 9 aortas at 6 weeks in the cell group; and none of the aortas in the medium group.

Conclusion: The results show that grafted neonatal cardiomyocytes survive, differentiate, develop a blood supply and spontaneously contract within the outer wall of the aorta in rats. It is feasible to transplant immature cardiac cells into the aorta to fashion an external auxiliary circulatory pump.

9:30 a.m.

870-5

Uncovering Human Cardiac Myocyte Progenitor Cells for Myocardial Regeneration

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Background: The failure to identify and culture human cardiac progenitor cells has reinforced the idea that the heart is a terminally differentiated organ, with little or no capacity for self renewal. Increasing evidence suggests baseline levels of myocyte loss in the

human myocardium require sustainable myocyte renewal for cardiac homeostasis. We investigated whether cardiac progenitor cells can be cultured from the human myocardium.

Methods: Cells derived from cultured human right atrial tissue explants (RATEs) were depleted of fibroblast surface antigen (FSA) positive cells using immunomagnetic beads. The resultant FSA negative fraction was assessed for cells expressing markers of cardiac differentiation using flow cytometry and confocal microscopy as well as for stem cell markers and the cell cycle antigen Ki-67.

Results: Human atrial myocardial tissue explants produce a heterogeneous cell population amongst which are small highly proliferating cells, expressing markers of cardiac differentiation; Csx/Nkx-2.5, GATA-4, alpha-sarcomeric actinin, cardiac myosin heavy chain together with the cell cycle antigen Ki-67. In addition the cells also express c-Kit and MDR-1 denoting stem cell properties. At 9-12 weeks in culture they fuse to form rod like multinucleate cells similar to those previously described as myotubes.

Conclusions: Our study shows for the first time that there is a potential for self renewal of the human myocardium involving cardiac progenitor cells and that cardiac stem cells can be cultured. This has major implications in the study of stem cell biology and the development of cell therapy for myocardial regeneration.

9:45 a.m.

870-6

The Role of Angiopoietin-1 in Physiologic and Pathologic Cardiac Remodeling and in Promoting Cardiac Myocyte Survival

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Background: Cardiac remodeling contributes to cardiovascular disease progression, emerging as a therapeutic target for heart failure. While remodeling initially compensates for injured myocardium, it is a progressive process that is ultimately maladaptive. Understanding processes resulting in adaptive growth are needed. In development, the heart grows and remodels via increases in myocytes (hypertrophy, hyperplasia) and capillaries (angiogenesis). We propose angiopoietin-1 (ang1), a vascular maturation factor, is a mediator of cardiovascular remodeling. Ang1 is required for cardiac development and increases in hypoxic myocardium. Ang1's receptor, tie2 is endothelial-specific, so its role is thought to be limited to vessels. A recent study shows that ang1 binds integrins, perhaps revealing new roles for ang1 in cardiac development and remodeling.

Methods: We measured ang1, ang2, and tie2 mRNA levels in developing/adult hearts (fetal, 1, 2, 4, 7, 14, 21, 56, 133, 238 d) and thyroid hormone (T3)-induced cardiac hypertrophy (1-4, 7, 14 d) using C57BL/6 mice, and in neonatal/adult cardiac myocytes and a cardiac myocyte cell line (C2C12) (real-time RT-PCR). We compared ang1/tie in the left ventricle (LV), right ventricle (RV), and atria (AT) (*in vivo* studies). We examined if ang1 binds (cell adhesion) and promotes cardiac myocyte survival/proliferation using *in vitro* studies (C2C12 cells).

Results: Ventricular and atrial ang1 levels increase in the fetal to adult transition. Ang1 increases (LV=475, RV=75, AT=4 fold) are related to the extent that the chamber mass increases. Ang1 was undetectable in neonatal, but prominent in adult cardiac myocytes. T3-induced cardiac hypertrophy increases LV ang1 up to 20 fold. Cardiac myocytes bind ang1. Ang1 markedly promotes myocyte survival and proliferation in serum-free media (7 d versus controls < 1 d) with the number of surviving cells declining from 7-21 d.

Conclusion: Our studies support a role for ang1 in physiologic and pathologic cardiovascular remodeling. They identify a mechanism for enabling myocyte survival/proliferation in adverse conditions, and have broad implications for cardiac hypertrophy/heart failure therapy development.

ORAL CONTRIBUTIONS**872 Heart Failure: Resynchronization Therapy**

Wednesday, March 10, 2004, 8:30 a.m.-10:00 a.m.

Morial Convention Center, Room 260

8:30 a.m.

872-1

Reverse Remodeling With Cardiac Resynchronization Therapy Varies With Infarct Location: Analysis of Echocardiographic Data From the MIRACLE Trial

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Reverse remodeling has been reported in moderate to severe heart failure (HF) patients (pts) with ventricular dysynchrony after cardiac resynchronization therapy (CRT). This analysis assessed whether infarct location predicted the degree of reverse remodeling after 6 months of CRT. **METHODS:** The MIRACLE study enrolled NYHA Class III/IV HF pts with ventricular dysynchrony and LVEF≤35%. All pts were implanted with an InSync atrial synchronous biventricular pacing system. AV delay was individually optimized and pts were randomized to no pacing (control) or to CRT. Doppler echocardiograms were analyzed by a core laboratory. Left ventricular end diastolic volume (LVEDV), LV end systolic volume (LVESV), LV ejection fraction (LVEF), and mitral regurgitation color flow jet area (MR) were calculated. Infarct location was determined using a 16 segment model. Anterior infarction (AMI) was defined as akinesis in at least 3 antero-apical segments and inferior infarction (IMI) defined as akinesis in at least 3 postero-inferior segments. Changes in LV remodeling were quantified and compared between the AMI and IMI groups after 6 months of CRT. **RESULTS:** Significant reductions in LVEDV and LVESV